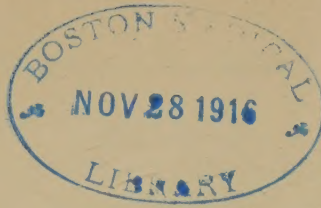


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THE MESONEPHRIC CORPUSCLES OF THE SHEEP, COW, AND DEER

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THREE FIGURES

In this paper I have employed the name corpuscle to indicate the entire blind end of an excretory tubule, the term glomerulus to indicate the knot of capillaries, covered by the inner wall, projecting into the expansion of this end. A corpuscle (Malpighian) then includes ordinarily a glomerulus, the slit-like crescentic cavity continuous with that of the tubule, and the outer layer or Bowman's capsule.

The renal and mesonephric corpuscles of mammals have been so long known and their development so fully studied that a new type, not yet described to my knowledge, seems to me worthy of a short note. Most of the investigations on this subject have been carried out in man, rabbit, and pig, and the similarity of the corpuscles in these forms, in shape and in developmental history, to those of birds and reptiles probably discouraged wider research in other classes of mammals. The peculiar corpuscles to be described are found in the sheep, cow, and deer, and thus may be typical of the ruminants. They are not mentioned in the Normmentafel of the deer by Sakurai.¹

The excretory corpuscles and tubules develop, as is well known, from the nephrogenic portion of the nephrotome. The most detailed work on the changes in this tissue which lead to the formation of the corpuscles is that of Stoerk,² who studied the renal corpuscles in man, and of Felix³ who described the meso-

¹ Keibel's Normmentafel, Reh. 1906.

² Anat. Hefte, Abt. 1, Bd. 23, 1903.

³ Keibel and Mall's Human Emb., vol. 2, p. 796 et seq., 1912.

nephric corpuseles in man and rabbit. According to both these authors the nephrogenic tissue first is divided or gathered into separate solid masses or balls, which then become hollow rounded vesicles. These elongate to a tubular form, and the cavity of one end becomes T-shaped, by means of an increased thickness of the epithelium along the tube, thus narrowing its lumen (the upright bar of the T), and a lateral expansion of the end of the vesicle with a flattening of the end wall (the transverse bar of the T). Later one side of the transverse bar is exaggerated at the expense of the other, and this together with a curve in the opposite direction at the other end of the upright, which opens into the Wolffian duct, gives to the tubular vesicle the shape of the letter S. The blind expanded end becomes flattened, and in the hollow develops a knot of blood-vessels, which with further growth is enveloped in a double-walled cup. The glomerulus then appears suspended in a rounded cavity by a slender neck, through which its vessels pass. The inner wall of the cup closely invests the capillaries, which are arranged in lobules, and its epithelium is modified so as to present thin plates in contact with the endothelium of the blood-vessels. The outer wall of the cup, Bowman's capsule, remains relatively undifferentiated as a single layer of flat cells resting on connective tissue. The slit-like, crescentic cavity of the cup is continuous with the lumen of the tubule, and at the junction the flat epithelium of Bowman's capsule changes to the cuboidal epithelium of the tubule wall.

Slight variations from this normal type have been noted. MacCallum⁴ found by injections that in the Wolffian body of older pig embryos the efferent glomerular arteries, of which there may be many, "usually proceed from the side opposite the entry of the afferent vessels;" this indicates either a very broad neck or base from which the glomerulus projects, or the division of the neck by the encroachment of the cavity, as happens in the perforation of the mesocardium. Fused corpuseles and instances where two or more tubules lead from a

⁴ Am. Jour. Anat., vol. 1, no. 3, 1902.

single cavity have also been described. In the first case the outer wall, Bowman's capsule, of two or more neighboring corpuscles have fused and then degenerated, leaving two or more glomeruli suspended in a single cavity from which two or more tubules arise; in the second case it is probable that after the fusion of the cavities all but one of the glomeruli have disappeared, making the fused cavities appear like a single one. In petromyzon Wheeler⁵ has described the actual fusion of several glomeruli, separate at first, into a single large glomus, with many separate afferent and efferent vessels. But in all these cases a distinct glomerulus, projecting into and nearly filling the cavity, is present, and in all there is a distinct Bowman's capsule.

In the sheep the posterior corpuscles of the fully formed Wolffian body correspond in all essential details with those of birds, reptiles, and the previously studied mammals. In the more anterior portions a different type presents itself. If a tubule here is followed from the Wolffian duct toward its blind end it is found to open ultimately into a rather wide cavity, with a nearly circular wall in the transverse plane, but with the horizontal walls flat and nearly parallel. The neighboring cavities are in relation to each other by their flat walls, the top of one making the bottom of the other. (Figure 1. In transverse sections the two types do not appear strikingly different.) Frequently the cavities are wedge-shaped in sagittal section, the dorsal and ventral walls being of unequal length. Into this cavity, which represents the crescentic, slit-like cavity of the usual mesonephric corpuscle, no large single glomerulus protrudes; instead the top and bottom walls are provided with a close network of capillaries, some projecting slightly in little tufts. The endothelial cells are covered by the epithelium lining the cavity, here again taking the form of thin plates, as in the true glomeruli. The vessels in these horizontal walls supply the surface of the cavities above and below, so that in sagittal sections each septum shows an irregular border on each side.

⁵ Zool. Jahrb. Abt. Anat., Bd. 13, 1899.

In the anterior end of the gland the horizontal walls of these separate cavities are wanting in places, and become more and more irregular. A large cavity with many partially closed compartments results, from which open many tubules. As the incomplete horizontal walls retain the same character as those of the separate cavities, with vascular tufts projecting on each side, they might easily be mistaken, in sagittal sections, for long narrow glomeruli; but the lack of any neck and the presence of a connective tissue core reveal the true condition. This large irregular cavity bears the same relation to the closed cavities below as do the fused corpuscles already mentioned to the more typical ones.

It will be seen that in these compartments Bowman's capsule does not exist as such, and can only be represented by those portions of the walls free from the protruding capillaries. In these places, where the lining epithelium rests on the subjacent connective tissue, the epithelial cells are flat, but without the extremely thin plates, and not in direct relation with the few blood-vessels present. The largest areas of this type are on the perpendicular circular walls, where the capillary tufts are fewest. The flat epithelium changes to cuboidal at the opening of the tubule.

The large fused cavity represents the corpuscles of the anterior fifteen to twenty tubules; the separate, individual cavities, opening each into a single tubule, number about eight or ten. The ordinary corpuscles begin then at the 23rd to the 30th tubule, and extend throughout the remainder of the gland. The point where the anterior type ceases is marked roughly by the groove on the genital ridge between the anterior and middle thirds, i.e., between the part destined to form the rete and that to form the sex gland proper; and it is with the anterior corpuscles that the rete establishes relations. Also at this point, near the ventral edge of the organ, there is an especially prominent cross connection of the mesonephric veins. At the line of demarcation there are two or three corpuscles, apparently always present in the same form, which show, by the enlargement of certain tufts almost to the proportions of true glomeruli, a transition from one type to the other.

The difference between the two types of corpuscles lies in the fact that in the more usual type one side only of the original cavity comes into close relation with the blood-vessels, the other remaining undifferentiated as Bowman's capsule, while in the anterior corpuscles of the sheep all sides, but especially the cranial and caudal sides of the original cavity are equally in relation to the vessels and equally differentiated. The cause of this seems to me to lie in the precocious development of the corpuscles in the sheep, cow, and deer, at a time when the Wolffian ridge is neither long enough nor broad enough to permit their proper expansion. As can be seen in figure 2 the cavity of the corpuscle is quite large and distended while the tubule is of insignificant length and quite straight. This is the stage at which the transverse lines of the T should be extending cranio-caudally in the plane of the figure, according to Felix.⁶ "The transverse bar has a cranio-caudal direction. In the angle between the upright and transverse bars the glomerulus appears, sometimes lying on the cranial and sometimes on the caudal side of the upright; the part of the transverse bar with which the glomerulus is associated becomes much more strongly developed than the other part." The extension of the transverse bars in a cranio-caudal direction seems, in these animals, to be rendered impossible by the close crowding of the vesicles. In the earliest formed, more anterior vesicles the attempt at such an extension leads to the fusion and dissolution of the end walls of the transverse bars, and the confluence of the cavities. (Upper part of figure 2.) With slightly more room the cavities do not join but the transverse bars cannot develop, and each vesicle remains symmetrical. (Lower part of figure 2.) In both cases the capillaries, when they appear, may come into equally close relations with the cranial and caudal walls, and because of the restricted length of the transverse bars a single group of capillaries may be in relation with the cranial wall of one vesicle and the caudal wall of that next anterior.

Such an inhibition of the transverse bars does not occur in the lower part of the Wolffian body, where the vesicles are formed

⁶ Loc. cit., p. 803.

only after the Wolffian ridge has expanded; nor does it occur in the kidney where the nephrogenic tissue is widely scattered. The normal process of glomerulus formation, similar to that described by Stoerk and Felix, can therefore take place, as is shown in the caudal end of the Wolffian body of a deer embryo of 6.4 mm. (fig. 3), where the increased growth of one limb of the transverse bar, and the consequent formation of glomerulus and crescentic cavity can easily be seen.

From the standpoint of physiological function this type of corpuscle, without glomerulus, seems capable of greater efficiency than the more common one, because it lacks to a great extent the supposedly inactive surface of Bowman's capsule. The capillary tufts might become larger and closer together until they practically filled the cavity, and offered an enormous secreting surface in the aggregate. As the corpuscles are found, with a rather large central cavity and small capillary tufts, I doubt if the secreting surface nearly equals that of the true glomeruli with their deep lobulation.

PLATE 1

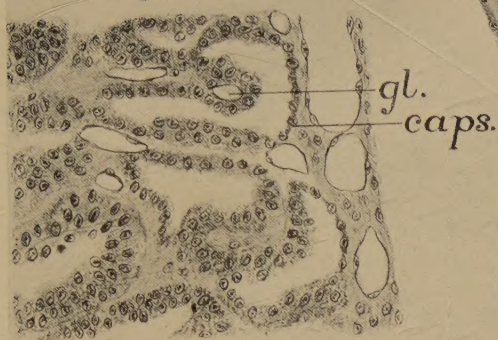
EXPLANATION OF FIGURES

1 Sheep, 14.6 mm. H.E.C., no. 1109, sect. 162. Sagittal section of the Wolffian body, lateral to the genital ridge, from about the 18th to the 32d tubule. The caudal six or seven corpuscles are of the ordinary type, the cranial ones of the special type. The fused corpuscles are not shown. *tr.*, transitional corpuscles with large capillary tufts; *gl.*, glomerulus; *caps.*, Bowman's capsule; *con. tub.*, convoluted tubule leaving corpuscle, others seen above, and again on dorsal (left) side of drawing; *ve.*, large cross connection of Wolffian veins, marking transition zone. $\times 96$ di.m.

2 Sheep, 5.0 mm. H.E.C., no. 1896, sect. 63. Sagittal section of the Wolffian body, from the 13th to the 19th tubule. Tubules 13, 14 and 15 have fused, making a large cavity continuous in another section with the anterior tubules. 17 and 18 are symmetrically expanded, but not fused, and open into the Wolffian duct at the left of the drawing. 19 is in the solid stage. *som.*, somite. Numbers refer to corpuscles. Note flat epithelium, comparable to that of Bowman's capsule at end of corpuscles. The growth of the tubules later rotates their junction with the corpuscles to the ventral side (*cf.* fig. 1). $\times 200$ diam.

3 Deer, *Cervus capreolus*, 6.4 mm. H.E.C., no. 1519, sect. 46. Sagittal section of lower part of Wolffian body, to show the asymmetrical extension of one side of the transverse bar, and the formation of the ordinary corpuscles. *Gl.*, glomerulus; *caps.*, Bowman's capsule. $\times 200$ diam.

Fig.3



som.

Fig.2

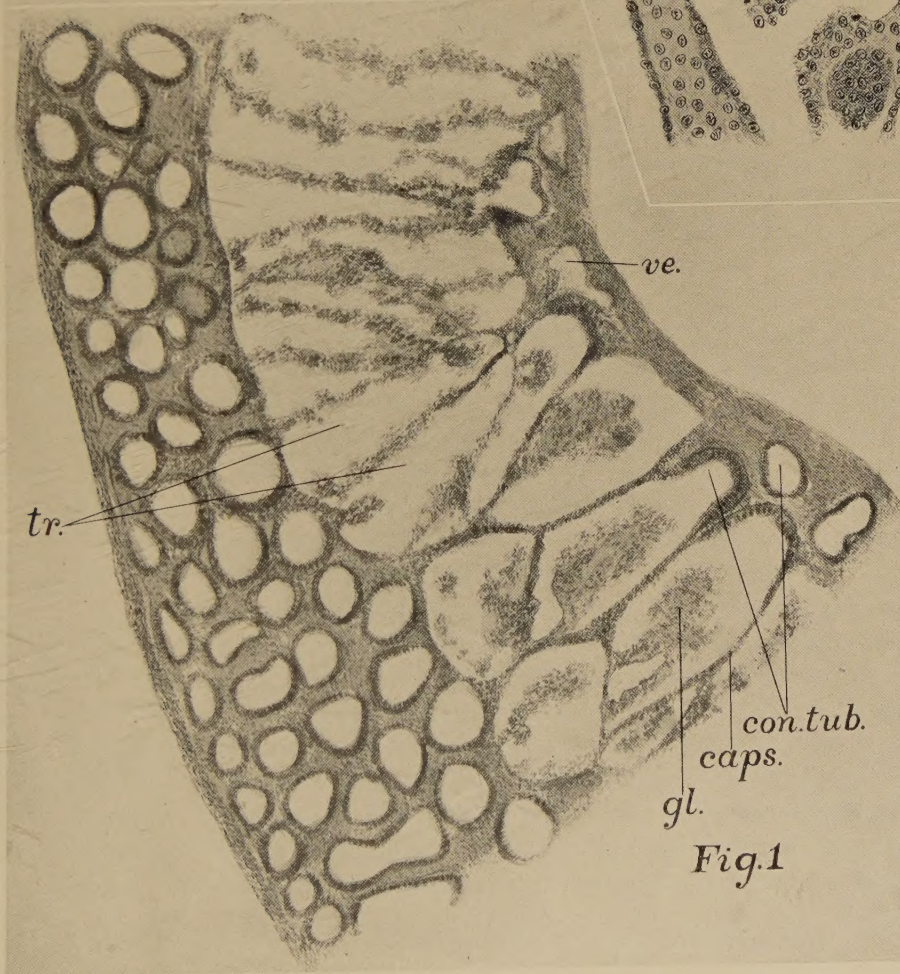


ve.

tr.

gl.
caps.
con.tub.

Fig.1



TRUNCUS ARTERIOSUS COMMUNIS PERSISTENS ✓

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ONE FIGURE

The comparatively uncomplicated defect which this case presents, dating from an early period of embryonic development, and the evidence it affords of certain developmental processes, seem to justify its addition to the already almost endless literature of cardiac malformations.

The development of the heart in question has been normal except for the complete absence of the septum aorto-pulmonale and the anomalies of the ventricular septum and valves of the common arterial ostium which this defect necessarily entails. In addition, as is common in these cases, there is no ductus arteriosus formed.

The organ, which came from a child of 5 months, is very large, weighing ca. 65 gm. The ventricular portion is broad, the apex not very well defined, the bulging of the ventricles hides the root of the truncus, especially on the right side.

The atria are normal, the foramen ovale is closed; two left pulmonary veins open by a short common trunk into the left atrium, the condition of the right veins can not be determined. The Thebesian and Eustachian valves are well formed.

In the ventricles, the walls are of approximately equal thickness, ca. 8 mm. The capacity of the left seems rather greater than that of the right, both ventricles communicate freely with the truncus, the left being perhaps the favored one. The mitral valve shows nothing unusual, the anterior and posterior papillary muscles being well developed. The tricuspid valve possesses three well-marked leaflets but the incisures between the cusps fall some distance short of the attached base of the valve. The papillary muscles and their attachments to the

valves may be considered normal. The anterior papillary receives a large, well-defined moderator band. From the septum below and in front of the ventricular defect arise three strong chordae, the papillary muscle of the conus—of Luschka,

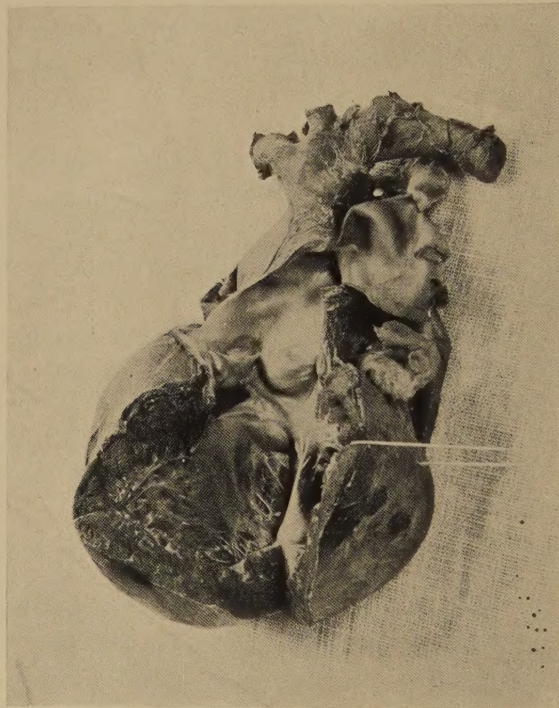


Fig. 1 Heart viewed from the left and in front. In the ventricle the medial cusp and posterior papillary muscle of the mitral valve are shown. The truncus and left pulmonary artery are opened up, the origin of the right pulmonary is also visible. Of the valves of the truncus only the right and posterior are well shown; below the former is the ventricular defect and behind this the intact pars membranacea. Through the defect can be seen some of the chordae of the anterior papillary muscle.

although the muscular part is but faintly indicated. These chordae pass to the adjacent ends of the anterior and medial cusps, and one crossing the medial cusp can be traced directly across the pars membranacea. The noduli Albini are very distinct on both mitral and tricuspid valves.

The large truncus communis arises almost equally from both ventricles, its origin, which is distinctly constricted, is hidden in the deep atrioventricular groove, being over-lapped particularly by the conus portion of the right ventricle. Distal to the semilunar valves it suddenly enlarges, especially toward the right, this wall being strongly convex while the left wall is practically straight. From the left side of the truncus, and rather nearer its origin than the concavity of the arch of the aorta, arises the left pulmonary artery. The right pulmonary artery arises at the same level from the dorsal wall of the truncus on left side, close to the left pulmonary. The right artery turns at once sharply to the right to assume its normal position behind the truncus. Both vessels are of equal calibre and much thinner than the truncus or the aorta. There is no trace of the ductus Botalli. No evidence whatever of the distal portion of the septum aorto-pulmonale is present, i.e., there is here a total persistence, as contrasted with cases of partial persistence, of the truncus arteriosus. Indeed the only feature indicating even an intended subdivision is the shifting to the left of the right pulmonary—of the right sixth arch. Beyond the pulmonaries the aorta decreases rapidly in size, its arch giving off the three usual branches. The semilunar valves of the truncus are three in number and perfectly formed. The cusps are so arranged that one is posterior the other two anterior, of these last two the left is more lateral than the right. From the corresponding right and left sinuses arise the coronary arteries, while above the posterior cusp is a slightly marked, transversely elongated depression, limited above by a faint ridge (indistinctly seen in the photograph) resembling an occluded vessel, but nothing can be seen externally.

The ventricular defect appears as an elongated slit between the upper border of the muscular septum and the lower surface of the truncal valves. Its lower and anterior limits are formed by the muscular septum and heart wall, behind it is bounded by the anterior concave margin of the pars membranacea. The pars membranacea—not including the septum atrioventriculare, is a thin, translucent membrane, devoid of muscular fibers

and triangular in outline. Its posterior rounded apex is situated slightly below the center of the posterior truncal valve, its base or anterior margin is free and sharply concave, running out below onto the muscular septum while above it is lost in the interval between the right and posterior semilunar valves, being confluent with, or giving attachment to, the anterior leaflet of the tricuspid valve. The left side of the membranous septum is easily seen, the right forms the mesial boundary of a small pocket which is limited laterally by the medial, and to a slight extent by the anterior leaflet of the right venous valve. It is on this surface that the above mentioned chorda is found. Between the upper attachment of the free border of the membranous septum and the right semilunar valve, and close to the latter, is a small nodule resembling the noduli Albini. The pars membranacea is much larger than usual and, as far as can be ascertained now, is bent over to the right along its attachment to the muscular septum, so that its left surface looks upward and is brought close to, if not in contact with, the right half of the posterior semilunar valve, particularly during closure of the truncal valves.

The conus part of the right ventricle is well developed, forming a deep recess above and between the tricuspid orifice and the ventricular defect. Externally this recess is seen as the prominent bulging on the ventral surface of the heart which covers the root of the truncus. The cardiac wall is here semi-translucent, covered with trabeculae which reach almost to the semilunar valves, and is the thinnest portion of the ventricular wall. An undoubted crista supraventricularis cannot be identified, although there is large trabecular mass, forming the right boundary of the thin-walled conus, running up to the right truncal valve, much like the trabeculae which have a similar relation to the left and more especially to the right cusp of the pulmonary valve in the adult.

Viewed from the right ventricle all of the right semilunar valve can be seen, nearly half of the posterior and a small portion of the left. During distension the valves would apparently quite fill the interventricular opening.

The specimen under consideration offers a concrete confirmation of our views of the development in the region of embryonic auricular canal; views which, although by no means new, still do not seem to be universally current. With a complete failure of development of the septum aorto-pulmonale and the subsequent interventricular communication, there is, nevertheless, a well-formed pars membranacea septi, indeed the interventricular portion of the membranous septum is more extensive than usual. The interventricular opening in this case—a defect in the posterior part of the anterior septum according to Rokitansky—has nothing whatever to do with the pars membranacea, but is due to the non-development of the septum aorto-pulmonale, aggravated it may be by some consequent arrest of development in the muscular septum. The great majority of interventricular foramina, regardless of the condition of the septum aorto-pulmonale, do not implicate the membranous septum for the simple reason that the only relation of the two septa is topographical and they are almost as independent in their development as they are in their origin. The entire pars membranacea septi, atrio ventricular as well as interventricular portion, and the major part if not all of the atrio ventricular valves are derivatives of the endothelial cushions of the primitive auricular canal, and the adult relations reproduce essentially those of the embryo. Hence their common histological characters in the adult and the intimate relations between the membranous septum and the tricuspid valve. The fused right ends of the anterior and posterior endothelial cushions give rise to the mesial part of the anterior cusp and to the entire medial cusp of the tricuspid valve, while toward the left these same cushions furnish material for the entire pars membranacea although as a rule most of the interventricular portion may be a derivative of the posterior cushion. Evidence for this is provided by the chordae which pass from the membranous septum to the anterior and medial cusps. Although these cusps are as a rule not entirely distinct, cases can be found where the adjacent tips of the leaflets are in no way connected but run out on the pars membranacea leaving a distinct interval

between them. Granted the origin of the valves from the anterior and posterior cushions, one could, in the cases just cited, determine the relative extent of the membranous septum derived from each cushion. In the heart here described the subdivisions of the valve leaflets are not sufficiently marked to determine accurately their relation to the septum. In view of the foregoing one can understand the rarity of pure, uncomplicated defects of the membranous part of the inter-ventricular septum while not denying (Rokitansky) their occurrence.

The development of this heart has been normal except for the septum aorto-pulmonale, even the enlarged conus portion of the right ventricle being present. The blood would leave the right ventricle in the direction of the pulmonary arteries but would be hopelessly mingled with that issuing from the left side.

The heart, as shown in the photograph, was obtained through the kindness of Dr. V. C. Rowland of this city and was the only material available for study so that nothing is known as to the presence of anomalies elsewhere.

A DOUBLE UMBILICUS

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THREE FIGURES

After reviewing a considerable literature on anomalies and being unable to find such a case reported, it is deemed worth while to put this specimen on record.

The anomaly here described was found in a colored man, estimated to be about 55 years of age, during our regular class work in anatomy. The surface anatomy of the abdominal wall was normal, except for the presence of a small subcutaneous tumor a short distance above the umbilicus which was supposed to be a lipoma. On removal of the skin a sharply circumscribed knob of fibrous tissue was found in the subcutaneous tissue 4 cm. above the umbilical fovea and 0.5 cm. to the right of the mid line (fig. 1). This knob of tissue was 2.5 cm. in diameter and 0.7 cm. thick. It was rather soft in consistency and was definitely surrounded by a thin capsule of connective tissue, except posteriorly where it was continuous with the round ligament of the liver. Superficially it was continuous with the subcutaneous tissue though it separated easily, while its deep surface much more dense merged imperceptibly into the obliterated umbilical vein. The latter entered the abdominal cavity through a foramen in the aponeurotic wall of the abdomen. The foramen had a smooth margin unconnected with the wall of the vein; so that the vein could glide easily backwards and forwards through the foramen. On section the knob mentioned above was somewhat loose in texture and strands of tissue running in various directions gave it a lobulated appearance.

On opening the abdomen the urachus and obliterated hypogastric arteries were found extending up on the abdominal wall throwing up their usual folds of peritoneum. The urachus which

was apparently single became very small in the region of the umbilicus and seemed to terminate in the lower (true) umbilicus. The obliterated hypogastric arteries were of about the normal size and in the umbilical region two or three fibrous cords were given off on each side, which terminated in the lower (true) umbilicus while the remainder passed on to the upper (false) umbilicus (fig. 2).

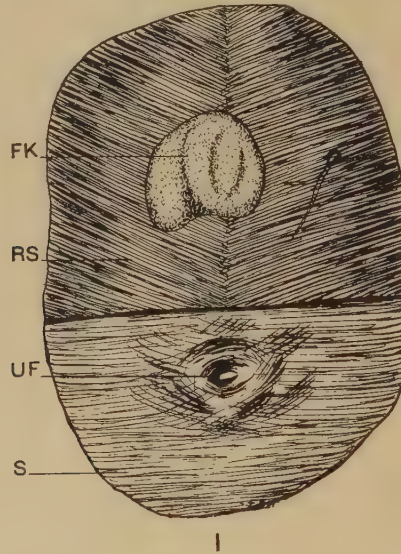


Fig. 1 *F.K.*, Fibrous Knob; *R.S.*, Rectus Sheath; *U.F.*, Umbilical Fovea; *S.*, Skin.

The obliterated umbilical vein was double, one being attached to the lower umbilicus while the other was somewhat larger and very firmly attached at the upper umbilicus to the fibrous knob previously described as being outside the rectus sheath in the subcutaneous tissue (fig. 2). After a very short course, however, the two fused forming the round ligament of the liver which extended upward enclosed in the lower margin of the falciform ligament.

Microscopic examination of the fibrous knob mentioned above revealed it to consist of rather dense connective tissue and a good deal of fat.

Regardless of the apparent weakness of the abdominal wall, it should be noted that there was little possibility of an umbilical hernia. In fact the arrangement was so unique that such could not occur (fig. 3), for the reason that whenever the intra-abdominal pressure was increased, the pull of the round ligament

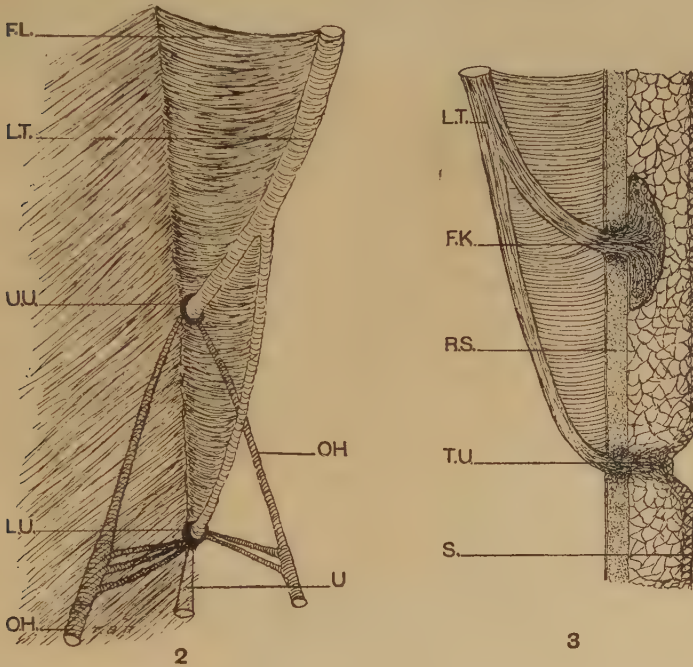


Fig. 2 *FL.*, Falciform Ligament; *LT.*, Ligamentum Teres; *UU.*, Upper Umbilicus; *LU.*, Lower Umbilicus; *OH.*, Obliterated Hypogastric; *U.*, Urachus.

Fig. 3 *LT.*, Ligamentum Teres; *FK.*, Fibrous Knob; *RS.*, Rectus Sheath; *TU.*, True Umbilicus; *S.*, Skin.

on the fibrous knob was in the same proportion, thus ensuring the weak area to be closed at all times.

To sum up: There entered the abdomen through the upper navel one umbilical vein and the terminal branches of both hypogastric arteries while the urachus, the other umbilical vein and branches of the hypogastric passed through the lower umbilicus.

When one recalls the changes taking place on the ventral wall of the embryo in very early embryonic life, it seems reasonable to suppose that such a condition as here described would happen much more frequently than it apparently does. An abnormal growth of tissue across the mid line between the allantois and yolk sac, dividing the single foramen into two, would seem to be the most probable cause of the condition. The anomalous fibrous cords on the posterior wall according to this explanation represent anastomoses between the vitelline and umbilical vessels. It is possible that the fibrous knob on the end of the umbilical vein represents the remnants of the umbilical vesicle. The fact, however, that its structure is directly continuous with the obliterated vein makes it seem more likely that it was merely a localized hypertrophy on the end of that vessel.

THE RELATION OF THE CHORDA DORSALIS TO THE ENTODERMAL COMPONENT OF THE HYPOPHYSIS

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From the Department of Anatomy, University of Michigan

TWELVE FIGURES

The mode of termination of the anterior end of the notochord has been variously interpreted by authors. The older writers thought the chorda to present a causal relation to the head flexure of the embryo; to act mechanically in drawing out the infundibulum of the pituitary from the brain wall; or to form, after a similar manner, the entodermal diverticulum known as Seessel's pouch. Later writers, while not necessarily arguing for a causal relationship, have noted the existence of contacts between notochord and hypophysis and between notochord and fore-gut. Recently the rôle of the entoderm in the formation of the hypophysis has occupied the attention of a number of observers. It is the object of this communication to call attention to an observed relation of the chorda to the entodermal component of the hypophysis and to attempt the explanation of certain connections of chorda and hypophysis.

An exhaustive review of the literature covering the development of the hypophysis will not be here attempted. For a very complete bibliography on this subject the interested reader may be referred to Stendell's monograph in Oppel's *Lehrbuch* and to the recent contributions of Bruni and Woerdeman (1914). Neither will the much-perturbed question of the origin of the notochord receive attention. For a theoretical study of this question a recent article by Triepel may be consulted. Only the literature which seems especially pertinent will be referred to.

The Entodermal Component of the Hypophysis. Rathke, whose name is used in designating the epithelial pouch from which the main body of the hypophysis arises, thought the structure to be derived from the anterior end of the fore-gut and thus to be entodermal. It was not until in 1873 that the now commonly accepted view of the ectodermal

origin of Rathke's pocket was established. Then, in rapid succession, came the contributions of Goette, Mihalkovics and Balfour stating that the pocket is 'pinched off' from the mouth invagination just anterior to the oral plate. As Balfour points out, Rathke's early mistake (shared by His, W. Müller and others) is undoubtedly due to the fact that he examined embryos in stages after the rupture of the oral membrane—"when it would naturally be impossible to tell whether the pinching off was from the epiblast of the mouth invagination or from the hypoblast of the throat."

The most recent writer to maintain that the glandular portion of the pituitary develops entirely from entoderm is Prather, who describes *Amia calva*. Prather says: "Thus, I repeat, there can be detected no fold or overgrowth of ectoderm to give rise to the hypophysis, nor overgrowth of the entoderm. Its cells are differentiated in situ apparently by longitudinal division of the cells constituting the roof of the fore-gut." Prather's contentions have not been widely accepted. Previously, the hypophysis in *Amia* had been stated by Dean to arise from the ectoderm. In the same year that Prather's communication appeared Reighard stated emphatically that the Amian hypophysis has its origin entirely from ectoderm. Gregory for the same form, states that the origin is both ectodermal and entodermal. P. E. Smith has attacked the problem anew and comes to the conclusion that the anlage is ectodermal, but adds that "it can be said with considerable probability that the entoderm contributes to the composition of the hypophysis." For other classes of the vertebrate kingdom many observers have recorded the existence of a more or less important entodermal component of the hypophysis.

Kupffer has described a growth from the cephalic end of the fore-gut to meet the dorsal wall of Rathke's pocket. This condition he interpreted as an attempted recurrence of an older pre-oral mouth, or paleostoma. He considers that the entoderm makes important contribution to the epithelial lobe of the hypophysis.

St. Remy, in studying chick embryos at about the seventieth hour of incubation, saw a communication between the cavities of Seessel's and Rathke's pouches. This singular communication, which appears to be of short duration, shows itself as an extremely fine orifice piercing the inferior part of the epithelial partition between the two invaginations. Seessel's pocket produces a solid bud which acquires a fine lumen. The bud persists until about the sixth day and then atrophies completely. "So the pharyngeal pocket of birds is to be considered as the representative of the entodermal part of the hypophysis, but only as a rudimentary one which takes no anatomical part in its formation."

Nusbaum has observed (dog embryos: 9 mm, 14 mm, 15-16 mm) that Seessel's pouch grows forward and meets the hypophyseal pocket. The lumen is obliterated in this part. This solid cord later becomes segmented into two or three cell groups lying close to one another, "von denen die oberste und ansehnlichste mit der hinteren und unteren

Wand der blasenartigen Erweiterung der Hypophyse innig sich verbindet. Diese Zellgruppe geht später, wenigstens teilweise, in die Wand der wachsenden Blase selbst über, während der Rest der ungewandelten Seessel'schen Tasche allmählich spurlos verschwindet." Nusbaum agrees with Kupffer in regarding these appearances as a degenerated recurrence of a pre-oral mouth.

Valenti, leading a number of Italian observers, has described an 'ecto-entodermal fusion' in the chick, somewhat similar to that seen by St. Remy. However, Valenti does not describe a communicating orifice. In certain amphibia he notes an entodermal cord, located some distance caudal to the mouth region, with which the cephalic end of the chorda is in close relation. This entodermal cord later takes on a semilunar form and contributes to the origin of the epithelial lobe of the hypophysis.

A comparative study has recently been made by Bruni in an attempt to homologize the entodermal components of the hypophysis. According to this observer in all the amniotes the entoderm contributes to the formation of the hypophysis in three different ways:

a) By an incorporation of Seessel's pouch (remains of the anterior end of the fore-gut). This contributes in that it helps to form a 'vestibulo faringoipofisario' located at the buccal border of the hypophyseal sac. It is later reduced to form a 'peduncolo faringoipofisario.'

b) By a diverticulum which is located between Rathke's and Seessel's pockets, and which may be called the 'diverticolo medio.'

c) By a solid bud which arises from the top of Seessel's pouch—'gemma della tasca di Seessel.'

Bruni's work will be again referred to in considering certain of my own findings.

Relations of Chorda to Hypophysis. W. Müller and Dursy state that the anterior end of the chorda is attached to the base of the fore-brain. It aids mechanically in drawing out the infundibular process. Other early observers held the opinion that the chorda is responsible, in a similar manner, for the drawing out of the hypophyseal sac. Mihalkovics denies both of these contentions. For the rabbit he states that the chorda, immediately after the rupture of the oral membrane, to use his own words, "zieht, die blinde Bucht des Vorderdarms umkreisend, bis an den oberen Stumpf der durchgerissenen Rachenhaut und endet ganz nahe beim Hornblatte." Later the chorda constantly touches the ectoderm at the dorsal wall of the forming hypophyseal sac. In denying that the chorda is responsible for the drawing out of the hypophyseal sac Mihalkovics calls attention to the fact that if such were the case the chorda should be attached to the upper angle of the hypophysis. This is indeed true in bird embryos, he states, but in the rabbit the notochord is attached to the inferior half of the dorsal wall of the hypophysis. This attachment is soon lost by the ingrowth of connective tissue.

Aside from attachments between chorda and hypophysis, which were thought to assist mechanically in forming the organ, Reichert (1840)

and His (1868) considered the hypophysis to be derived from the anterior end of the notochord. Dursy (1868) states that the chorda contributes the vascular stroma of the hypophysis, while the epithelial and nervous parts he considers as coming from the fore-gut and brain, respectively.

Koelliker (for a rabbit embryo of 11 days) has noted as proceeding from the inferior part of the dorsal wall of the hypophysis a structure which he describes in these words: "ein dicker zapfenförmiger nach hinten gerichteten Vorsprung von 76μ Dicke (Länge), der auf das vorderste Chorda ende zugeht und wie mit demselben zusammenzuhängen scheint, bei genauer Untersuchung jedoch durch eine Spalte von etwa 2μ von demselben getrennt ist." This 'chordazapfen' of the hypophyseal wall is composed of the same kind of cells as the dorsal wall and shows no demarcation from it. Koelliker takes this union as indicating an ectodermal origin for the notochord.

Woerdeman, observing embryos of *Sus scrofa* corresponding in age to Nos. 71 and 78 in Keibel's Normmentafel, has seen a true contact between the chorda and the dorsal wall of Rathke's pocket. He believes that he is justified in calling it a true contact because at the place of union there is no membrana propria intervening between the two structures; at this place there is a very noticeable thickening of the dorsal wall of Rathke's pocket; and the arrangement of the nuclei is very irregular. Woerdeman has noted, in connection with these observations, that Seessel's pouch has lost its lumen in the upper part and that it is directed toward the insertion of the chorda into the hypophyseal wall. He notes a similarity to Nusbaum's observations wherein a strand of cells from Seessel's pouch was seen to become attached to the dorsal wall of the hypophysis. In comparing this with his own findings Woerdeman's words read:

Ist dies nun auch bei *Sus scrofa* der Fall, was meines Erachtens nicht unmöglich sein wird, aber welches zu constatieren ich nicht das Glück gehabt habe, dann kann es wichtig sein, die Stelle zu kennen wo die Verbindung stattfindet, da mir die Möglichkeit nicht ausgeschlossen zu sein scheint, das die Rathkesche Tasche, die Chorda dorsalis und das Seesselsche Säckchen in eine sehr innige Beziehung zu einander kämen.

Relation of Chorda to Entoderm. That the notochord is attached to the entoderm in the mid-line at an early stage seems commonly acknowledged. Keibel firmly maintains that this union is secondary in the guinea-pig and also in the rabbit. This contact between chorda and entoderm persists until a short time after the rupture of the oral plate. The last point of contact is not at the stump of the oral plate but somewhat more caudal. With the final cutting off, to quote Keibel, "entstehen Bilder, welche Selenka zu der aufstellung seiner Gaumentasche geführt haben." Keibel denies that the formation of the chorda has any connection with the hypophysis or oral plate, either directly or mechanically.

For the chick, Lillie, in his text-book, speaks thus briefly on the early connection of chorda and entoderm:

The head process becomes inseparably fused with the entoderm in the middle line immediately after its formation; and this fusion is continued back along the axis of the primitive streak. The fusion is particularly intimate and persistent at the extreme anterior end of the head process; behind this point the notochord and entoderm soon separate again in the course of development. But the anterior end of the notochord remains attached to the entoderm for a considerable period after the formation of the head-fold.

Selenka has described a peculiar 'gland-like' structure in the embryonic opossum. In its complete state this 'Gaumentasche,' as he calls it, consists of a pouch with 2-4 hollow or solid out-pouchings. The pocket empties into the fore-gut; dorsally it is in direct communication with the substance on the notochord. Selenka states: "Die Gaumentasche ist mithin nichts Anderes als das vordere ausgehöhlte Ende der Chorda, eine vordere Chordatasche." This investigator has found a similar structure in the higher vertebrates and believes that it exists in all forms. He thinks that Seessel's pocket cannot be the 'Gaumentasche' for in both chick and duck embryos he has observed that the 'Gaumentasche' is already closed and its opening into the pharynx constricted by the time the hypophyseal sac has begun to form. According to him Seessel's pocket is only a mechanically modified remains of the fore-gut.

In a brief note, Bawden has described what he interprets as Selenka's 'pharyngeal sac' in the duck and finds that it is not branched as in the opossum. It is best developed in the duck embryo of five days and is transitory, leaving no trace behind it after the seventh day. In some stages the sac has been noted to become incorporated into the stalk of the hypophysis.

St. Remy (1896) after a wide study of the mode of termination of the anterior end of the chorda concludes that in all the amniotes the chorda bends itself to maintain its insertion into the epithelium, forming an angle more or less pronounced. One can distinguish an ascending part, the termination of the principal part of the chorda, and a descending part attached to the entoderm. The latter disappears constantly very early through the disintegration of its elements, which transform themselves into connective tissue. The summit of the angle and a part of the ascending branch disappear likewise.

Mrs. Gage has noted the existence in the human embryo of several points of contact between notochord and epithelium of the pharyngeal roof, caudal to Seessel's pocket. In an extended comparative study this condition was not found to be common except that it exists in a certain percentage of cases in the pig. As a summary of the comparative study Mrs. Gage states that the cephalic tip of the notochord may vary in relation, and may be in contact with the hypophysis, Seessel's pocket, or the first mesodermic head cavity. She suggests

that the last condition, which is found in the shark, may be the typical one.

Recently Huber has treated the relation of the chorda to the median pharyngeal recess or the pharyngeal bursa. His article may be consulted for a review of the work of Froriep, Killian, Levi, Meyer, Grünwald and others having a bearing on this point. Huber controverts the statement of R. Meyer that the pharyngeal bursa is formed from Seessel's pouch. He finds (human embryos) that the chorda is in constant relation with a diverticulum from the pharynx. The region of this contact is separated from Seessel's pocket by almost the entire future roof of the pharyngeal cavity.

MATERIALS AND METHODS

The material for this study consists of about forty rabbit embryos obtained during the first twenty days of development, and of sixteen chick embryos ranging in age from 36 to 120 hours of incubation.

The rabbit embryos, which are from the embryological collection of the Department of Anatomy, University of Michigan, were fixed in Zenker's, Carnoy's or Bouin's fluid and cut in the three dimensions. Of these series ten were especially prepared for a closely allied study and were cut into sagittal series of 5μ thickness—the younger ones on the rotary microtome, and the older ones on the sliding microtome by Huber's water-on-the-knife method. The staining was done for the most part with iron-alum hematoxylin and Congo red.

The chick embryos are partly from the author's collection and partly from the embryological collection of the Department of Anatomy, University of Michigan. They were fixed in Zenker's or Carnoy's fluid and were cut into sagittal series at 5μ and 7μ . Some are stained with iron-alum hematoxylin and Congo red and others with hemalum and Congo red.

In a study of the development of the hypophysis in the rabbit a number of models were prepared after the Born wax-plate method, to show the hypophysis region, including Seessel's pouch and the anterior end of the notochord. These were made at a relatively high magnification— $400\times$ for the younger stages and $200\times$ for the older ones. The models clearly show the relations existing between the anterior end of the notochord,

Seessel's pouch and Rathke's pocket. Similar models have been made of certain of the younger chick embryos at a magnification of $300\times$.

For the purposes of this study mid-sagittal outline drawings were reconstructed by superimposing the drawings from several consecutive sections. This corrects for any slight obliquity of cutting and presents in a single picture what could be seen as such only in a thick, exactly central section. In every instance the relations shown by the reconstructed outline drawings are substantiated by the wax-plate reconstructions.

RELATIONS OF THE CHORDA IN THE RABBIT

The earliest stages of rabbit embryos at my disposal show the notochord attached to the entoderm of the fore-gut, for a considerable distance caudal from its cephalic extremity. Figure 1 shows a mid-sagittal drawing of a 3 mm rabbit embryo. The oral membrane is still intact and Rathke's pocket exists only as a shallow invagination. The notochord is separated from the entoderm of the fore-gut forward to a region indicated by the X. From this point to the oral plate is a comparatively long region of contact still existing between notochord and entoderm, although in a number of places dissolution of this contact has begun. The anterior end of the notochord is rounded and is in close relation to the dorsal wall of the hypophysis invagination, but no actual point of fusion can be demonstrated. A small diverticulum extends cephalo-ventrally from the cavity of the fore-gut close to the oral plate but this may be, in part at least, an artefact due to an inbuckling of the oral plate during fixation.

A somewhat older embryo, with the oral membrane in the process of rupture, shows the notochord losing its contact with the entoderm at several places around the anterior end of the fore-gut.

A still older embryo (5.5 mm) shows notochord and entoderm entirely separated except for one point just ventral to the tip of the fore-gut. A mid-sagittal drawing of this embryo is shown in figure 2. Rathke's pocket has deepened considerably and its previously regular contour is being modified by the pushing in

of the infundibular process. Immediately caudal to Rathke's pocket is Seessel's pouch or pocket, derived from the blind anterior end of the fore-gut. It is about one-half as deep as Rathke's pocket and has a somewhat wider opening into the cavity of the fore-gut. The boundary between the two pockets is well marked

Fig. 1 Reconstructed outline drawing, in mid-plane sagittal section, of head region of rabbit embryo, Series A, 3 mm crown-breech length. $\times 30$. Notochord black, brain wall shaded. *R*, anlage of Rathke's pocket; *S*, Seessel's pouch (anterior end of fore-gut); *OM*, oral membrane. Notochord shown in contact with entoderm from point marked X forward to dorsal attachment of oral membrane.

Fig. 2 Reconstructed outline drawing, in mid-plane sagittal section, of hypophysis region of rabbit embryo, Series D, 5.5 mm crown-breech length. $\times 30$. Notochord black, brain wall shaded. *R*, Rathke's pocket; *S*, Seessel's pouch. Cephalic end of notochord directed toward a bud of entoderm from near apex of Seessel's pouch.

Fig. 3 Reconstructed outline drawing, in mid-plane sagittal section, of rabbit embryo 7 A, 15 days of development. $\times 30$. Notochord black, brain wall shaded. *H*, hypophysis; *S*, remains of Seessel's pouch. Cephalic end of notochord in proximity to bud of epithelium from Seessel's pouch.

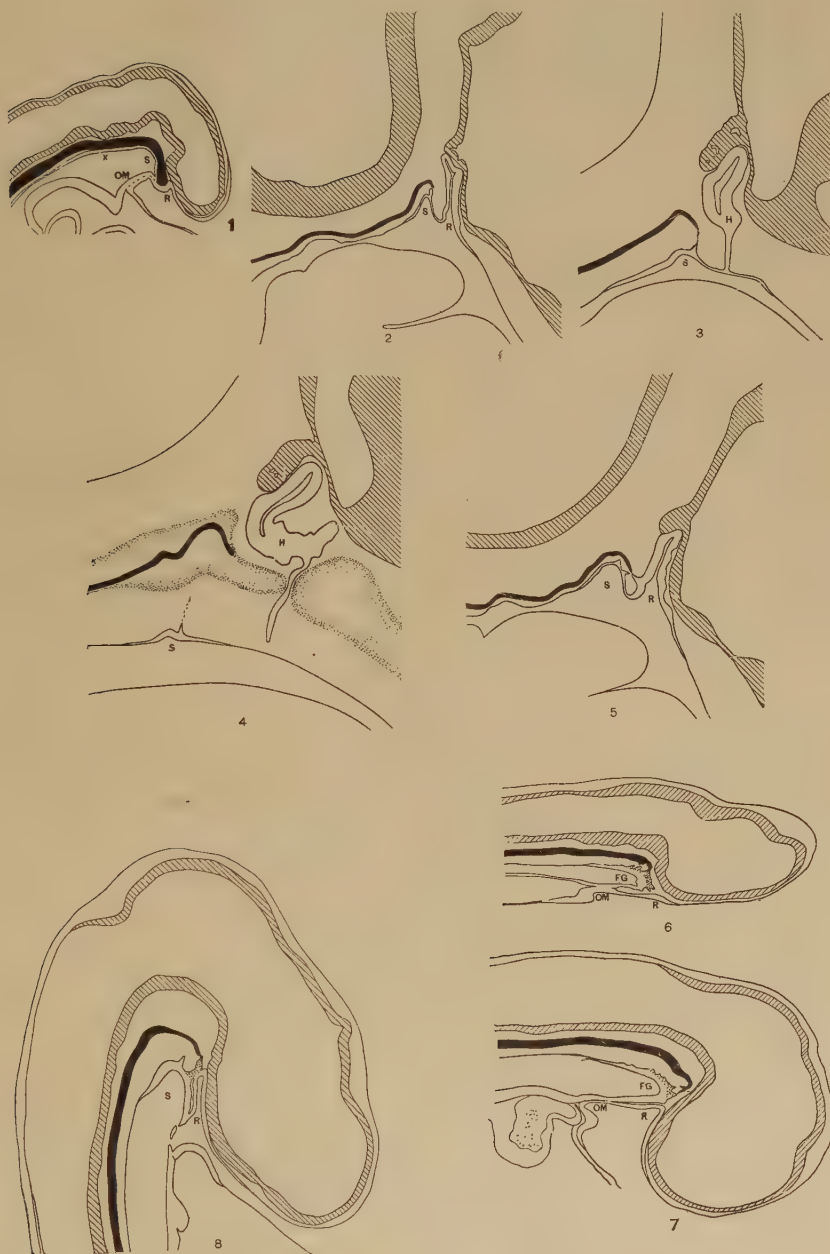
Fig. 4 Reconstructed outline drawing, in mid-plane sagittal section, of hypophysis region of rabbit embryo 8 A, 16 days of development. $\times 30$. Notochord black, brain wall shaded; cartilage mass of skull base stippled. *H*, hypophysis; *S*, remains of Seessel's pouch. A bud from Seessel's pouch is directed toward the cephalic part of the notochord.

Fig. 5 Reconstructed outline drawing, in mid-plane sagittal section, of hypophysis region of rabbit embryo, Series F, 6 mm crown-breech length. $\times 30$. Notochord black, brain wall shaded. *R*, Rathke's pocket, *S*, Seessel's pouch. Cephalic end of notochord in relation to buds from both Rathke's and Seessel's pouches.

Fig. 6 Reconstructed outline drawing, in mid-plane sagittal section, of head region, chick embryo of 19 somites. $\times 30$. Notochord black, brain wall shaded. *FG*, fore-gut (later Seessel's pouch); *R*, anlage of Rathke's pocket; *OM*, oral membrane. Cephalic end of notochord attached to anterior end of fore-gut; evidences of recently severed connections more caudal. Portions of entoderm in contact, or recently in contact, with notochord stippled.

Fig. 7 Reconstructed outline drawing, in mid-plane sagittal section, of head region, chick embryo of 22 somites. $\times 30$. Notochord black, brain wall shaded. *FG*, fore-gut (later Seessel's pouch); *R*, Rathke's pocket; *OM*, oral membrane. Cephalic end of notochord still attached to anterior end of fore-gut by epithelial bud (stippled).

Fig. 8 Reconstructed outline drawing, in mid-plane sagittal section, of head region, chick embryo of 29 somites. $\times 30$. Notochord black, brain wall shaded. *S*, Seessel's pouch; *R*, Rathke's pocket. Oral membrane in process of rupture. Cephalic end of notochord attached to epithelial bud (stippled) from Seessel's pouch; bud now fused with dorsal wall of Rathke's pocket near apex.



and there is no reason to doubt that the oral membrane had its upper attachment fairly between the two as they now exist. On the ventral side of the angle of Seessel's pouch appears a solid epithelial bud. The notochord follows the configuration of the entoderm as far forward as Seessel's pocket, where it dips down and comes into close relation with the solid bud of epithelium just described. An actual contact does not appear to exist, the two structures being separated by a space of 5μ .

Numerous other embryos, closely staged, show the notochord in a similar close relation to a solid bud of epithelium, always in the same relative position. Gradually Seessel's pouch is reduced until it is represented only by a slight indentation of the roof of the bucco-pharynx. The reduction of the size of the epithelial bud is not always proportionate, so that it is often relatively prominent. A drawing of a 15-day rabbit embryo is presented in figure 3. The hypophysis shows connection with the epithelium by a solid cord of cells. Seessel's pouch is found in the same relative position as in preceding embryos but is now much flattened. A well-marked epithelial bud extends from the ventral side of the pouch near its apex. This bud, arising from the epithelium by a broad base, tapers rapidly so that its apex is drawn out into a fine point. This point is directed toward the cephalic termination of the notochord, which makes a marked ventral bend and comes in proximity to the end of the epithelial bud. Between the two is a line of cells more densely arranged than the surrounding mesenchyme. The end of the notochord shows signs of degeneration: the boundary of its extremity is not well marked, large spaces occur in it, and the cytoplasm of most of the cells stains heavily with Congo red. The appearance presented warrants the interpretation that this is the last region of contact between notochord and entoderm.

By degeneration of the end of the chorda and of the epithelial bud, and by a rapid increase of connective tissues to form the skull base, the notochord becomes farther removed from any connection with the entoderm. A rabbit embryo of 16 days is shown in figure 4. The chondrocranium is well indicated at this stage and the solid stalk of the hypophysis has lost its

attachment to the epithelium of the bucco-pharynx. Seessel's pouch is represented by little more than an undulation of thickened epithelium. In the same relative position as in younger embryos, however, is found a solid bud. It terminates in a fine point. Extending for some distance from the end of the bud is a line of cells darker than the surrounding mesenchyme. The notochord, which more caudally is seen to lie in the ventral part of the basioccipital cartilage, cuts through the body of this cartilage mass, and after making two marked flexures ends close to the cavity of the future sella turcica. The end of the notochord shows signs of degeneration.

In rabbit embryo, Series L, which is almost exactly the same age as the embryo just described, a much more prominent epithelial bud occurs, but it is situated in the same relative position. In the case of this embryo the end of the bud is not drawn out to a sharp point but is rounded off and has a well-defined boundary. Likewise the notochord does not show degenerative processes at its end. Its termination is rounded up and ball-like, with a definite outline. Thus in some embryos there may appear for a time a retardation of the degeneration of the chorda which normally manifests itself quite early.

The process of separation of notochord and entoderm as here briefly outlined is confirmed by a careful examination of the many intermediate and corresponding stages composing the material for this study. In only two embryos (Series F and 6A, 13 days) was any indication of a connection between notochord and hypophysis noted. These two embryos are close together in stage of development and present much the same appearance. One of them (Series F) is shown in figure 5. Here it is seen that the end of the notochord encircles Seessel's pouch and is directed toward an outbudding from the inferior part of the dorsal wall of the hypophyseal pocket. Toward the tip of this bud is directed a bud from Seessel's pouch. The ends of the notochord, of the ectodermal bud and of the entodermal bud are all drawn out to fine points. No absolute connection can be demonstrated between any two of them but they are all three in close relation. This was believed by Woerdeman to be the case in the pig,

although he admits his lack of material to demonstrate their "innige Beziehung zu einander."

From the scarcity of stages in the rabbit showing any indication of connection between notochord and hypophysis I am led to believe that a union of these two structures, when it does occur, is accidental rather than of normal occurrence. Figure 1 shows that in early stages the end of the notochord lies close to the dorsal wall of the forming hypophyseal pocket. The presence of the notochord is responsible for an indentation of this dorsal wall showing that considerable pressure occurs between the two. It is not inconceivable, then, that a fusion may occur in a certain number of cases.

On the other hand the constancy of relation between the notochord and an epithelial bud from Seessel's pouch is noteworthy. This bud of epithelium appears constantly a short distance ventral to the top of Seessel's pouch. It is probably to be considered identical with Selenka's 'Gaumentasche' (solid in higher forms), and St. Remy's 'branche descendante' of the notochord.

RELATIONS OF THE CHORDA IN THE CHICK

My observations on chick embryos, beginning with a stage of 17 somites, are in accord with the generally accepted view that the notochord loses its attachment to the entoderm first caudally, then gradually forward. A mid-sagittal view of a 19 somite chick is shown in figure 6, and a view of the wax reconstruction made of the hypophysis region of this chick is shown in figure 9, A. The hypophysis anlage is indicated by a region of thickened epithelium. The connection between notochord and entoderm is lost except at the anterior end of the fore-gut. However, the entoderm shows evidences of this recently-severed connection by a considerable region of thickening anteriorly and by several buds just caudal to the present connection. There is no connection between notochord and hypophysis anlage, nor between the latter and the entoderm of the fore-gut.

An older chick (22 somites, figs. 7 and 9, B) shows the hypophysis as a well marked angle with a broad opening into the oral invagination. The notochord shows an attachment to a drawn

out portion of the entoderm at the anterior end of the fore-gut and presents evidences of other connections more caudal. The hypophyseal anlage is not connected with either notochord or entoderm.

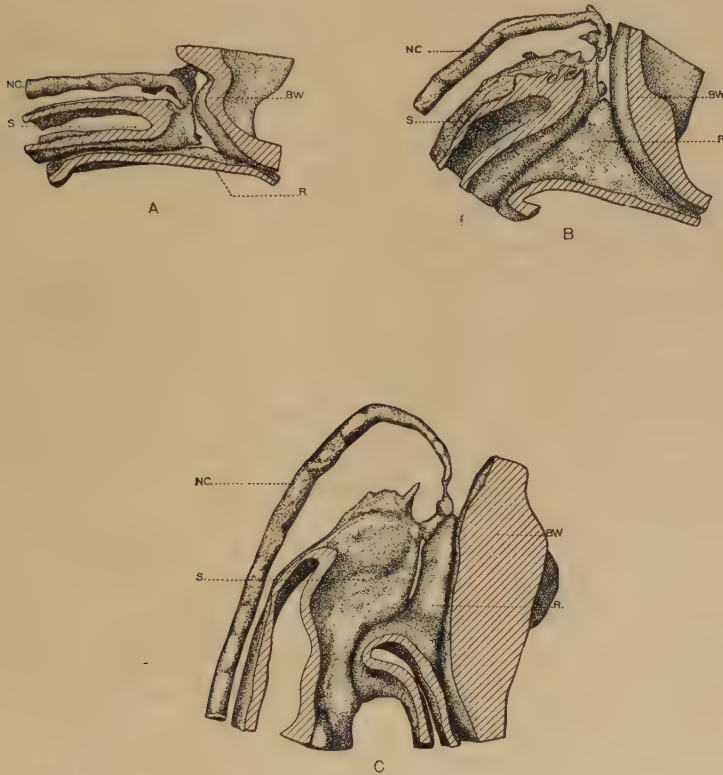


Fig. 9 Wax models of hypophysis region of chick embryos. $\times 75$. *A*, chick of 19 somites; *B*, chick of 22 somites; *C*, chick of 29 somites. The embryos from which these models were made are shown in mid-sagittal section in figures 6, 7 and 8, respectively. *A* and *B* show cephalic end of notochord attached to bud from anterior end of fore-gut. In *C* this bud has become fused with superior part of dorsal wall of Rathke's pocket. *NC*, notochord; *S*, Seessel's pouch (anterior end of fore-gut); *R*, Rathke's pocket; *BW*, brain wall.

An embryo with the oral membrane in process of rupture (29 somites, figs. 8 and 9, *C*) shows a marked increase in the size of the fore-brain vesicle which has resulted in a compression of the oral invagination and a flattening of the hypophyseal

pocket. But the most noteworthy result of this compression has been the bringing together of Rathke's pocket and the epithelial bud from the entoderm which is apparently further drawn out by its persisting connection with the notochord. The bud, proceeding from the entodermal epithelium at a point slightly ventral to the cephalic end of the fore-gut, lies in connection with the ectodermal epithelium of the dorsal wall of Rathke's pocket for a considerable region near its blind end. The connection is a true fusion of entoderm and ectoderm with no demarcation between the two. The notochord makes a bend to maintain its connection with the entodermal bud. It will now be noted that the end of the notochord is nearer to the wall of Rathke's pocket than to the fore-gut (later Seessel's pouch). Here notochord, ectoderm and entoderm are in close relation. However, the notochord does not touch Rathke's pocket directly but only by means of the epithelial bud through which it maintains its connection with entoderm. At the anterior end of the fore-gut is another entodermal bud directed toward the notochord. This is to be considered as one of the remains of the earlier, more general union between notochord and entoderm.¹

A strikingly similar condition is presented by another embryo of about the same age (latter half of the third day of incubation). The hypophysis region is shown in figure 10. The oral membrane has not broken but is very thin. A bud from the entoderm lies fused with the superior part of the dorsal wall of Rathke's pocket for some distance. The result of this fusion is to more than double the thickness of the hypophyseal wall in this region. The notochord shows an unquestionable contact with the end of the entodermal bud by a dense line of cells, which is considered to be the anterior end of the notochord in process of disintegration.

Three other embryos, with the oral membrane in various stages of rupture, show distinctly this fusion of entoderm and

¹ Such appearances are not uncommon, some embryos presenting two or even more such structures, which may persist later than the rupture of the oral membrane.

ectoderm with the remains of the anterior end of the notochord in relation to the place of fusion. These five chicks cover quite well the latter part of the third day of incubation, yet in

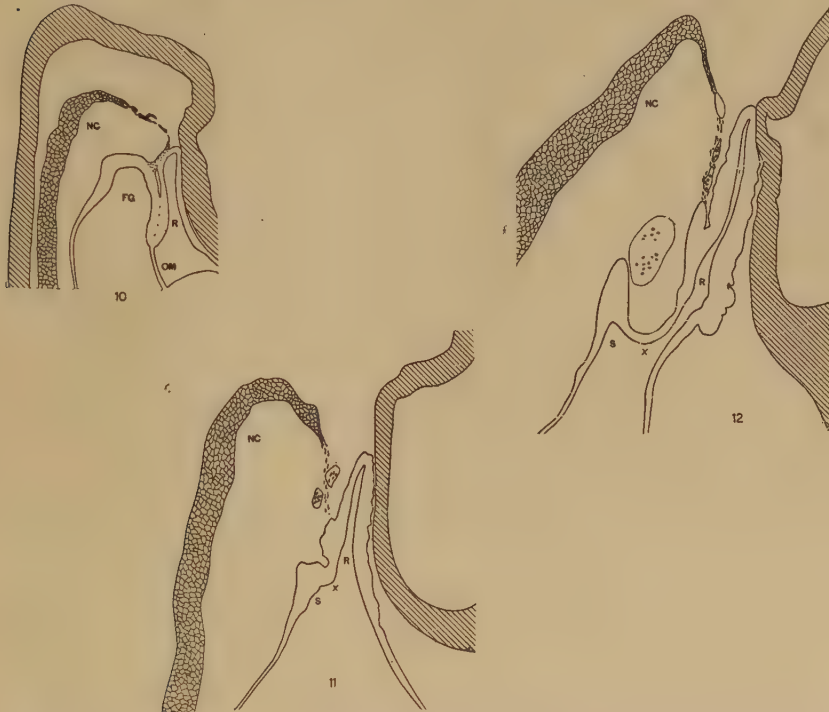


Fig. 10 Reconstructed outline drawing, in mid-plane sagittal section, of hypophysis region of chick embryo in latter half of third day of incubation. $\times 55$. Brain floor shaded. *NC*, notochord; *FG*, fore-gut (later Seessel's pouch); *R*, Rathke's pocket; *OM*, oral membrane. Shows same relations as figure 8.

Fig. 11 Reconstructed outline drawing, in mid-plane sagittal section, of hypophysis region, chick embryo, 96 hours of incubation. $\times 55$. Brain wall shaded. *NC*, notochord; *S*, remains of Seessel's pouch; *R*, Rathke's pocket; *X*, transition from ectoderm to entoderm. Fusion between Seessel's and Rathke's pockets now lost. A line of cells connects cephalic end of notochord to a bud from dorsal wall of Rathke's pocket.

Fig. 12 Reconstructed outline drawing, in mid-plane sagittal section, of hypophysis region, chick embryo, 120 hours of incubation. $\times 55$. Brain wall shaded. *NC*, notochord; *S*, remains of Seessel's pouch; *R*, Rathke's pocket; *X*, transition from ectoderm to entoderm. Large blood vessel shown between Seessel's and Rathke's pockets. Notochord shows traces of an attachment to a bud from dorsal wall of Rathke's pocket.

none of them could be found the fine communication between the cavities of Rathke's and Seessel's pouches as described by St. Remy.

In older stages the fused entodermal bud loses its connection with the fore-gut but remains attached to the ectoderm of Rathke's pocket and causes a thickening of a small portion of its dorsal wall. With this thickening the notochord shows a connection which gradually becomes less definite as its cells are drawn out and separated (St. Remy maintains that they are changed into connective tissue). The hypophysis grows most rapidly at its blind end and thus the point of entodermal fusion (with the degenerated end of the notochord in relation) comes to lie relatively nearer to the mouth of the hypophyseal pocket. This has been noted by Woerdeman, who finds (pig) that in young embryos a fusion of notochord and the dorsal wall of Rathke's pocket is found near the apex of the pocket, while in an older stage the insertion of the notochord is into the middle of the dorsal wall.

A chick of 96 hours incubation (fig. 11) shows Seessel's and Rathke's pockets in communication in the mid-plane. On the dorsal wall are found two epithelial thickenings. From the more cephalic of the two a line of cells leads to the end of the notochord. This would seem to indicate that this thickening is the location of the former ento-ectodermal fusion. The thickening is bud-like and may be what Bruni calls the 'diverticolo medio.' It is worthy of note that while the outermost part of this thickening is entodermal (remains of the bud that fused with the hypophyseal wall), yet none of this entoderm need be supposed to lie next to the lumen of the hypophyseal sac. At this stage it is rather difficult, from mid-sagittal sections, to determine the boundary between Rathke's and Seessel's pouches. An examination of more lateral sections indicates that this is at the point marked X (fig. 11). The epithelial thickening caudal to this point is the tip of Seessel's pocket. Bruni calls this the "gemma della tasca di Seessel."

A chick of 120 hours incubation (fig. 12) shows much the same relations but Seessel's pouch has again assumed a more definite

demarcation from Rathke's pocket. This is due to growth of mesoderm dorsal to Rathke's pocket and especially to the development of a prominent blood vessel growing between the two. This vessel has been spoken of by several authors as a connecting branch between the internal carotids, common in bird embryos. The notochord still shows indications of a connection with a bud (which sometimes shows a lumen) from the inferior part of the dorsal wall of Rathke's pocket. This must be Bruni's 'diverticolo medio.' Small blood vessels occur in this location. If the interpretation of the point X as the transition between ectoderm and entoderm is correct, then the hypophysis proper contains no entodermal component except the relatively few cells fused with the dorsal wall at about the time of the rupture of the oral membrane. It is hoped that the fate of Seessel's pouch may serve as the subject of a future study.

COMPARISON OF RELATIONS IN RABBIT AND CHICK

In comparing conditions found in the rabbit with those in the chick it is seen that in both the anterior end of the notochord tends to maintain a connection with the fore-gut, as St. Remy states. This connection is aided by a bud drawn out from the epithelium. In the rabbit the bud remains for some time, gradually decreasing as the epithelium of Seessel's pouch flattens out. In the chick the rapid growth of the fore-brain and the sharpness of the cervical flexure bring this bud into contact with the growing hypophyseal wall and the two become fused. Soon afterwards the bud loses its original connection with the entoderm but remains fused with the wall of Rathke's pocket. Thus a small mass of entodermal cells becomes united with hypophysis anlage. The notochord shows indications of a connection with this mass of entodermal cells which now form a bud on the dorsal wall of Rathke's pocket. By this means the attachment of the notochord is transferred from Seessel's pouch to Rathke's pocket.

In the rabbit, on the other hand, the notochord does not usually come into connection with Rathke's pocket. A fusion may occur in a small percentage of cases but must be considered

accidental. When such a union occurs a bud of entodermal epithelium may be directed toward the point of union.

These observations may be offered to explain certain connections between notochord and hypophysis as seen by Koelliker (in the rabbit) and Woerdeman (in the pig). The latter has in two cases, seen a more definite fusion than can be demonstrated in the two rabbit embryos which show evidences of such connection used in this study. These observations on the rabbit and particularly on the chick, may also serve to explain certain entodermal components of the hypophysis as noted by Kupffer (amphibia and mammals), Valenti (amphibia and birds), St. Remy (chick), and Nusbaum (dog). The findings here recorded, it seems to me, do not substantiate the view, taken by most of these writers, that the entodermal contribution to the hypophysis is of fundamental importance, especially phylogenetically; nor do they aid in the belief that the hypophysis represents a paleostome.

While the fate of Seessel's pouch and the participation of its epithelium in the composition of the hypophysis may be doubtful in the chick, it cannot be stated that this structure takes part in the formation of the hypophysis of the rabbit. As may be seen from the figures (1-5) Seessel's pouch is at all times sharply defined from Rathke's. This is especially well brought out in numerous wax reconstructions made from embryos closely staged before, during and after the rupture of the oral membrane.

For the rabbit it has not been possible to demonstrate a structure similar to the 'diverticolo medio' which Bruni describes for the rat.

CONCLUSIONS

For the rabbit:

1. In the rabbit the anterior end of the notochord tends to maintain its connection with the entoderm represented by a bud from Seessel's pouch.
2. The entoderm cannot be said to contribute to the formation of the hypophysis of the rabbit.
3. Usually the notochord does not come into connection with the wall of the hypophysis in the rabbit.

For the chick:

4. In early stages of chick embryos the anterior end of the notochord is attached to a solid bud of epithelium which extends from a point slightly ventral to the cephalic end of the fore-gut.

5. By the growth of the forebrain and the sharpness of the cervical flexure, this entodermal bud comes into contact with the growing hypophyseal sac and fuses with it. The fusion occurs at about the time the oral membrane ruptures.

6. The bud is solid; a lumen such as is described by St. Remy could not be demonstrated.

7. The fused bud soon loses its connection with the entoderm. It remains fused with the dorsal wall of Rathke's pocket, however, and contributes a small mass of cells to the hypophysis anlage.

8. The notochord still shows indications of an attachment to the fused bud. Thus the attachment of the notochord (which is gradually becoming less and less a definite attachment) is transferred from Seessel's pouch to Rathke's pocket.

9. The relation of the notochord to this small entodermal increment of the hypophysis, and the fact that no lumen is to be found, tend to disprove the view that the entodermal contribution is anything more than an accidental union of parts.

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ON THE CONVERSION OF A PHOTOGRAPH INTO A LINE DRAWING

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TWO FIGURES

The making of suitable drawings for reproduction has always been a problem, especially in laboratories which do not command the services of a competent artist. The modern recourse to a use of photography and photo-micrography with subsequent reproduction in half-tone, while greatly reducing the labor of preparation, has not produced pictures uniformly satisfactory in simplicity and definiteness. Wax models do not readily lend themselves to photographic methods. Photographs of anatomical dissections do not prove sufficiently clear for easy interpretation after having been subjected to the necessary reduction.

The process of line drawing on the other hand leaves nothing to be desired in clearness, since there needs to be depicted only what seems to be essential. Still the execution of suitable drawings from such subjects as those above mentioned requires considerable artistic ability and no little valuable time. As is well known the cost of reproducing photographs and drawings in half-tone is much greater than that of reproducing line drawings in black and white.

With these considerations in mind it has seemed that a means for more generally utilizing the line method would be welcome, if, at the same time, it might be possible to eliminate some of its demands on time and artistic skill.

At the suggestion of Dr. Huber a method which has been in use for many years by commercial artists and engravers, and known as the 'silver print', has been adapted to the use of scientific illustrators.

In brief the method is: 1) To produce a passable negative of the model, dissection or other object to be pictured. 2) Print from this negative an enlargement of suitable size on bromide paper.¹ 3) Trace outline of print with waterproof ink and fill in shaded portions by stippling, or by a few lines judiciously placed. 4) Bleach out all traces of photograph.

In making the initial negative only the ordinary precautions of careful lighting, proper orientation, etc., are necessary. The conceal-

¹ If the negative is large enough contact prints on cheaper papers, even blue prints, may be used instead of enlargements.

ing of supporting frames and wires is not required, nor will these need to be removed later by opaquing the background of the negative. A large negative is not necessary, 4×5 or 5×7 plates sufficing for most subjects, while for some even $3\frac{1}{4} \times 4\frac{1}{4}$ plates will be found large enough.

If the laboratory possesses a 'copying, enlarging and reducing' camera the printing of all sizes of enlargements up to and including 8×10 inches will be an easy matter. The camera is arranged as ordinarily used in making enlargements or reductions. The spring which usually holds the plate firm in the plate-holder should be removed. The bromide paper is then inserted and supported at the back by one or more sheets of stiff cardboard. If the bromide paper is of fair weight no difficulty will be experienced in keeping it flat and during exposure it may be handled precisely as a dry plate. Of course it will be necessary to select a bromide paper with a smooth surface so that it will later take the pen readily.²

When the print has been allowed to dry thoroughly it may be inked in, using of course waterproof India ink. The coarseness of the lines must be proportionate to the amount of reduction which the drawing is later to undergo. At all events the lines and dots must not be too close together. It is best not to try to reproduce all the detail of the photograph.

After allowing the ink to dry, the print is placed first in a tray of water for a few minutes and then transferred to a tray containing the following two solutions in the proportion of eight parts of No. 1 to one part of No. 2.

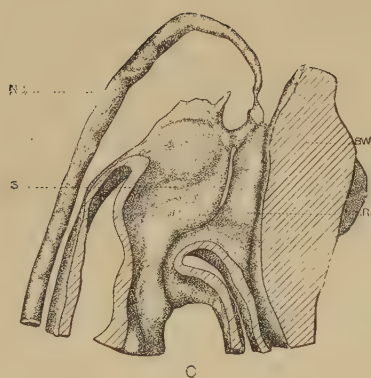
1. Hypo (thiosulphate of sodium), 30 g.; water, 480 cc.
2. Potassium ferrieyanide, 30 g.; water, 480 cc.

The print should remain in this bath until the last traces of the photograph proper have disappeared, which may require from 20 to 30 minutes. It is best to do this in a dark room. Then after washing for 15 or 20 minutes in running water, the drawing may be dried flat or mounted on card board. Some care is necessary in handling the drawing while wet so as not to rub the surface for the ink is then soft enough to smear.

This method results in the production of a clean drawing, accurate in outline, and with the shade correctly placed so that perspective should be properly brought out.

The accompanying figures show a model of the hypophysis region of a chick embryo reproduced by both half-tone and line etchings, the latter prepared by the method here described.

² This laboratory has successfully used the 'P.M.C.' bromide paper in 'Smooth' and 'Glossy' surfaces (Nos. 2 and 4) made by the Eastman Kodak Company.



A CORRECTION—R. W. SHUFELDT

At the time I was preparing my article on the "Comparative osteology of certain rails and cranes, and the systematic positions of the super-suborders Gruiformes and Ralliformes," which appeared in the October issue of *THE ANATOMICAL RECORD* (Vol. 9, No. 10, pp. 731-750, 1915), I had before me two manuscripts, namely the old one, published many years ago, when I considered that the *Aramidae* was a family belonging among the cranes and their allies (*Gruiformes*), and the remodeled one, in which my present views were set forth. In assembling the pages, the *old* page upon which the classification and some of the remarks under 'conclusions' appeared, was accidentally substituted for the *new* one carrying the new classificatory scheme upon it. In this shape it was handed over to be typewritten. When galley proof came to hand, I was extremely busy with other work, and it was therefore turned over to an expert proofreader and most carefully corrected. This proofreader knew nothing of the classification of birds, however, and so the galleys went forward, with the result now to be found on pages 749, 750. In so far as my present views are concerned, with respect to the position of the *Aramidae* in the system, they are correctly set forth in *THE ANATOMICAL RECORD* of August 20, 1915 (Vol. 9, No. 8, pp. 591-606).

PRESERVATION OF ANATOMIC DISSECTIONS WITH PERMANENT COLOR OF MUSCLES, VESSELS AND ORGANS BY NEWER METHODS

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The newer methods for the preservation of anatomic dissections are to be commended for retaining the color in the preparation and for the uniformity of results. They are classified as the Chemical Method and the Paint Method.

The subject must be embalmed with the following solution:

A—Arsenious Acid (Saturated Solution).....	2½ gallons
Potassium Nitrate pulverized.....	1 pound
Formol (must be white and clear, not greenish).....	6 ounces
B—Alcohol.....	16 ounces
Carbolic Acid, liquified (soluble).....	6 ounces
Glycerine.....	16 ounces
Creosote (Beechwood).....	2 ounces
Mix A and B; strain through a towel.	

One week after the embalming the vessels are injected with hot tallow, if desired. I use English vermilion deep shade for the arteries and ultramarine blue for the veins.

The further procedures vary according to the nature of the dissection, whether emphasis is to be given to muscles, viscera, or white tissues.

CHEMICAL METHOD

Preservation of color of muscles

It is not so very important to preserve the actual color of muscles, which varies from one subject to another, but it is essential that there should exist permanently a marked contrast between the fleshy parts of the muscles and the tendons, fascia, bones and other white tissues, which must remain white. A dark brown color of the fleshy parts of the muscles is satisfactory. The more red the brown is the better and prettier.

The selection of the subject is of great importance. None but lean subjects should be selected. The least fat makes an unsatisfactory preparation. Slim subjects with thin muscles do not make good preparations. Before embalming the subject, a cut three inches long

and one inch deep must be made in the deltoid or the external vast muscle, and the color of the muscles examined critically. If the muscles are of a very *dark* color they will make good preparations, but if the muscles are of a light pale color, they will seldom turn out satisfactorily. The method preserves the color presented by the muscles of the subject but seldom gives better color when the color is originally deficient. Negroes usually present darker muscles; also laborers. Subjects suitable for the work are not very common. Only about 8 or 10 per cent of the subjects which reach the Anatomical Department of Tulane are proper subjects. Each subject will yield about eight preparations of muscles and organs with satisfactory color.

The day after the injection with tallow the subject is cut up into the parts which are to be dissected. After which each part is placed in a large glass jar containing one per cent of soluble liquefied carbolic acid (C 1).¹ The solution must be changed as soon and as often as it becomes cloudy.

Muscles to be dissected, if not covered by skin, such as the muscles of the interior of the abdomen and the sternal region, should not go into C 1, since this solution destroys the color. They should be put up to drain at once, and dissected as soon as sufficiently drained, about 8 or 10 days.

When the solution remains clear or nearly so, the parts are transferred into an empty jar to drain and cure; hands, feet and knees up. The upper extremities must be fastened to a thin paraffined board to prevent their distortion. This stage should extend for about 4 or 6 weeks, during which time the color or contrast develops in the muscles. It is an important stage since the color depends much on it. The parts must be examined every week and if they show signs of hardening they must be placed in a 1 per cent solution of carbolic acid (C 1 or in F 1).

The parts are then dissected on a dissecting stand in the position

EXPLANATION OF ABBREVIATIONS

'C 1'—1 per cent of carbolic acid in filtered water.

'G 60 C 1'—60 per cent of glycerine with 33 per cent of water and 1 per cent of carbolic acid.

'CH. F 75'—a solution of chlorides with 75 per cent of formol and 25 per cent of filtered water.

'CH. F 20'—a solution of chlorides with 20 per cent of formol and 80 per cent of filtered water.

'CH. F. 5'—a solution of chlorides with 5 per cent of formol and 95 per cent of filtered water.

'G 30 C 1'—30 per cent of glycerine. 69 per cent of water and 1 per cent of carbolic acid.

'A 20'—20 per cent of alcohol in 80 per cent of filtered water.

'A 20 F 5'—20 per cent of alcohol, 75 per cent of filtered water and 5 per cent formol.

they are to occupy in the Museum. Not more than one layer should be shown in one dissection. The structures should not be raised from their beds.

The next step is to increase the color, to reinforce it because when later the preparation is put in the permanent solution the color will pale somewhat. Glycerine helps to fix the color, to increase it.

After the completion of the dissection it is placed for 24 hours in the following solution (G 60 C 1), each gallon of which is to contain:

Glycerine.....	66 ounces
Carbolic Acid, liquified.....	1 ounce
Filtered Water.....	33 ounces

This is about 60 per cent of glycerine. It registers 20 degrees with Baume Syrup Hydrometer. In this solution the muscle as also the tendons may assume a red color, but sometimes they become more or less black and shriveled, the preparations appearing as if they were ruined.

At the end of 24 hours, the preparation is placed in an empty jar with a lid. At the end of 30 days, when the glycerine has ceased to drip the preparation is taken out of the jar. The jar is washed and dried and there is placed in the bottom one-half pound of calcium chloride anhydrous (C. C. Mercks). The preparation is returned to the jar and the jar is closed with a lid, a rubber and a clamp. Some muscles darken very quickly, in a few days. They have to be examined daily. The tendons and muscles gradually darken and become as black as coal. The preparation again looks as if it had been ruined but later on everything rights itself.

When the preparation is thoroughly black, it is placed in filtered water with 75 per cent of formol (Ch. F. 75). The preparation remains in this solution for 24 hours, and is then placed permanently in a solution of chlorides of sodium, calcium and potassium, the proportions of which are given on another page. Twenty ounces of formol, clear and white, are added to each gallon of solution to prevent possible bacterial cloudiness. This is the Chemical or Chlorides-Formol Method (Ch. F. 20). It costs about 22 cents per gallon of solution. When immersed in the solution the muscles pale somewhat and that is why the muscles must be so dark before they are immersed.

Dissections presenting muscles with a light brown or pale color are unfavorable ones. They will seldom turn out satisfactorily. It is usually time, labor and material lost. This is an important point and should be well borne in mind to avoid disappointments. It is better to paint such preparations.

Preservation of the color of the viscera

Viscera, such as the lungs, heart, liver, spleen, kidneys, should as soon as dissected be placed directly in G 60 with one ounce of liquefied carbolic acid soluble (G 60 C 1). After 15 days in G 60 C 1, they must be transferred into Ch. F. 5.

The membranous viscera, mouth, nose, pharynx, oesophagus, stomach, intestines should be put directly in G 30 with one ounce of liquefied carbolic acid (G 30 C 1). After 15 days in G 30 C 1 they must be transferred into Ch. F. 5.

Preservation of color of the white tissues A 20 F. 5.

This method is suited only for dissections of white tissues: fresh bones (periosteum and marrow), articulations without muscular attachments, brain and membranes, spinal cord, pelvic organs in situ, larynx (cartilages and articulations), trachea, bronchi with or without lungs, eye-balls and sections, aponeuroses of the extremities and neck; dartoic tissues of penis, scrotum and pubis, testicles.

A gallon of solution contains:

Alcohol.....	20 ounces
Formol.....	5 ounces
Filtered Water.....	103 ounces

This solution contains about 15 per cent of alcohol and 4 per cent of formol. It is cheap, about 12 cents per gallon of solution. It is here designated as A 20 F 5.

The formol prevents the ivory-like bleaching action of the Alcohol, and the alcohol prevents the possible snow-white bleaching of the formol.

Special remarks

The proportions of the embalming solution are the result of many experiments. I have tried to increase the potassium, the formol and the carbolic acid, separately or conjointly, but with no satisfactory result.

Phenol is not exactly the same as soluble liquefied carbolic acid and should not be substituted for the latter.

The carbolic acid, formol and creosote are added to the arsenic to assist in the preservation, which the arsenic alone may not do thoroughly in warm weather.

The tallow as used for injection should be heated in a water bath to 210°F.

The connection of the syringe with the aorta or artery must be by tight double ligatures of white cord, not of a stiff material. Rubber tube connections must be wrapped with tape, to prevent bursting when injecting. The syringes must be previously tested with tepid water to make sure that the leather packing does not leak. When the

piston meets with a firm resistance no more pressure should be applied, lest rupture of vessels will take place and extravasation follow in the tissues.

Hot tallow injections of arteries is not always satisfactory as it does not always reach the small branches, such as the digitals and the labials. I have warmed the bodies in a tank up to 100° and above, but with not very much better results. I believe that the embalming fluid filling the smaller arteries prevents the tallow from reaching them. I have had no better results with other materials, gelatine, paint, etc.

When the subject is cut up the muscles present all sorts of color, raw, pale brown, dark brown, etc. But in the course of the next stages the muscles assume a more or less uniform color. However, in some subjects the muscles are irregularly colored. Those of a certain region may present a good dark brown color and those of another region a pale brown color. Sometimes the upper part of a muscle is satisfactory and the lower part of it is not as good. It is only when the parts are dissected that it can be positively ascertained what the color of the muscles is. Unless the muscles in the dissection present a very dark brown color, it is not likely that the ultimate color will be satisfactory.

When a number of preparations have been placed in the glycerine, this should be tested with the Baume Syrup Hydrometer. If it does not register B 20, glycerine should be added to bring it up to B 20 or it should be boiled down to B 20. It must be tested with the hydrometer when cold or about 60°F. It is a slow process, but quite a saving of glycerine. It is important to keep the glycerine at B 20.

When a number of preparations have been placed in the glycerine, and it has become very dark in color, in spite of filtering, it should be changed for a new solution.

The object of the immersion in glycerine is to lessen the blackening action of the calcium chloride, to which the preparation is exposed later on. If a preparation is exposed to the action of the calcium directly after it is dissected it will become so deeply black that it will remain so regardless of any solutions it may be put in.

When glycerine or other solutions mildew on the top or remain cloudy in spite of repeated filtering, there should be added to the solution ten ounces of alcohol to each gallon of solution.

The calcium is recovered by evaporation and when dry crushing it into small pieces. When the calcium liquefies, new hard calcium must be put in after drying the jar.

Solutions must be clarified or filtered as soon and as often as they become slightly cloudy or discolored. Keeping solutions clear all the time is the foundation of the preservation of the preparation. If allowed to stay in a cloudy solution the cloudiness will spoil the color of the colored tissues. If allowed to remain in a discolored solution, the white tissues in the preparation will become discolored. A five gallon percolator filter can be used for filtration.

In packing the percolator filter, a layer of three inches of clean white cotton is placed at the bottom, then a layer of four inches of first qual-

ity filter paper reduced into a pulp and pressed down; then a layer of five inches of granular bone black (3 pounds or half a gallon), the size of a millet seed. Before using the filter it should be thoroughly washed by pouring filtered water through it until the water comes out clear. Do the same each time the filter has been used, before putting in more solution to be filtered. The filtering must not be faster than fast falling drops.

When the filters are used for a different solution, they must be packed fresh, especially when solutions of G 60 and G 30 are filtered.

The bone black removes the color in the solution, but not the cloudiness. The filter paper removes the cloudiness but not the color.

Some of the solutions will remain hazy in spite of repeated filtering. They are clarified by putting in them five or ten grains of aluminium sulphate to the gallon of water. Stir well and filter. Repeat the aluminium, if necessary. Some solutions will not come out clear in spite of repeated filtering and the use of aluminium sulphate. A new solution should be fixed up to take the place. When the flushing water ceases to come out clear from the percolator, the percolator should be repacked.

The vessels should be painted before the preparation has been in calcium or in a solution. The vessels are wiped with gauze and then painted and the preparation is put in the solution. The same procedure applies to the affixing of the numerals to the structures. I use artists' paints, Chinese Vermilion for the arteries and dark blue for the veins. Water colors must be allowed to dry about 8 hours before the preparation is placed back into an aqueous solution, lest they will dissolve in it. In course of time the painted vessels may show yellowish spots or patches. They should be wiped off or scraped and the vessels repainted.

If preparations in Ch. F 20 darken too much in course of time, transfer them into A 20 F 5.

Do not use F 5 with G 60 or G 30.

There are now about 125 satisfactory preparations of muscles and organs made after these methods in the Souchon Museum at Tulane.

Some preparations do not turn out as good as they should and should be made over. Sometimes the same preparation has to be made over two or three times before a satisfactory one is reached. This happens almost solely in cases of dissections presenting a medium brown color. Dissections presenting a dark brown color seldom have to be made over.

The angle at which the light strikes the preparation has much effect in bringing out the color.

The numerals affixed to the structures are printed on ordinary paper with ordinary printer's ink, of the desired size, cut into circles or squares and stuck in places with tiny pins.

(The sizes of the jars needed for the work are Museum Jars (Whitall Tatum, Phila.) $24 \times 11\frac{1}{4}$; $24 \times 7\frac{1}{2}$; $36 \times 7\frac{1}{2}$.)

The Chlorides Formol Solution is composed as follows:

For a Jar containing One Gallon of Filtered Water:

Sodium Chloride (Commercial. Pure Table Salt).....	3 ounces
Calcium Chloride (Anhydrous, Merck's).....	15 grains
Potassium Chloride.....	1 drachm
Sodium Bicarbonate	45 grains
Formol.....	20 ounces

Stir and filter. The formol is to prevent the possible formation of bacterial cloudiness and also to preserve color.

It may be that 5 ounces of formol to the gallon will do as well as 20 and cheapen the solution. Experiments must determine that.

The chlorides fix the hemoglobin.

I am indebted to Professor Mann of Tulane for valuable information on chemical points.

THE OIL PAINT METHOD

The great advantage of the paint method is to utilize a number of preparations which upon the completion of the dissection do not present a satisfactory color and, therefore, are not suitable for the other method and would have to be thrown away. Dark brown or medium brown muscles are not as favorable for the paint method as muscles with a pale color.

Improved technic has yielded better results than on previous occasions. The oil paint method is a simpler and quicker method than the chemical method.

The subject must also be embalmed as described above.

After the completion of the dissection, the preparation is placed in a one per cent solution of Formol (F 1) for 2 or 3 days. It is then placed in an empty jar with a lid until all the water drips from it, about ten days.

The preparation is then mopped thoroughly dry with cheese-cloth and is painted first with gelatine, the next day with oil paint. When the gelatine is dry, in about 3 hours, another coat is given.

The gelatine solution consists of one leaf of white French gelatine to eight ounces of water dissolved by boiling. The gelatine is applied with a soft brush to the fleshy parts of the muscles only. The object of the two coatings of gelatine is to secure a smooth surface on which the paint will more easily and uniformly spread. It fills up the small cracks and holes in which the paint may gather and form streaks and spots. When the gelatine is dry, the muscles are painted.

The paint used for the muscles is French Carmine (Devoe), other carmines are apt to harden in the tube; for the arteries, Chinese Vermilion; for the veins, dark blue; for the portal system of veins, dark green; for the biliary ducts, chrome yellow. The mucous membranes (nose, mouth, pharynx, oesophagus, rectum, vagina, uterus) are painted pink (rose dore); the surfaces must be previously gelatined. The oil paints used are the artists' oil paints of Winsor and Newton, of London,

or Devoe of New York, purchasable in artists' materials stores. The brushes used are flat sable hair brushes, one-quarter of an inch wide, Devoe No. 8 and one-eighth of an inch wide, Devoe No. 4.

The paint is prepared by squeezing out of the tube one-third inch of French Carmine. Use the brush to prevent curling and to straighten out the little column of paint so as to better measure its length. Dissolve in a teaspoonful of rectified turpentine (Devoe). It takes some patience to secure the right color, dark raw beef meat.

Two thin coats or more are better than one single thick coat. Too thick paint makes unsatisfactory preparations. Too much paint cannot be wiped off after drying and the preparation is ruined. Sometimes one thin coat is enough if the muscles show a dark color.

When nearing tendons and fascias use a small brush with very little paint on the brush and no paint must reach the tendons and fascias.

The preparation must be painted a little darker than it is expected to look in the permanent solution because when immersed in the permanent solution the color becomes lighter. If the whole of the color or parts of it seems too light, the preparation is taken out of the solution and exposed to the air and mopped with cheese-cloth until dry enough for the paint to stick. Then another thin layer of paint is applied to those parts where it is too light. This may have to be done once or twice before the right tint is obtained. Should the carmine show too brightly add one-eighth of an inch of Vandyke Brown to tone it down. After the last coat of paint is applied the preparation must remain exposed to the air to dry, about 6 hours. The paint being an oil paint and being placed later in an aqueous solution need not be thoroughly dry before being immersed in the permanent solution. During the drying of the paint the tendons and the bones may assume a brown color from drying, but later on, in the permanent solution, they resume their white color.

The painted preparation must not be left exposed to the air overnight, lest it will dry too much. It should be placed for the night in an empty jar with a lid and taken out next morning to finish drying.

Before placing the preparation in the permanent solution, the lines between the various muscles must be marked with a knife to obtain definition.

When the paint is about dry, the preparation is place permanently in a solution composed of ten per cent of alcohol, and eighty-five per cent of filtered water (A 10).

Solutions must be changed or clarified as soon as they become the least cloudy or discolored.

Other good oil paints are house paints ready for use. Stir well before using. For muscles, use Tuscan Red No. 588 of Devoe Co. of New York one teaspoonful, with Maroon of True Tag Co. of Memphis one-half teaspoonful. Add three teaspoonfuls of rectified turpentine. These proportions are important. Two or three thin coats are better than one thick coat. Expose to the air to dry about six or eight hours

before placing in the permanent solution (A 10). They are hardier paints than the artists' Paints. They should be used to repaint artists' paints which unaccountably faded shortly or did not turn out good. I rather prefer them to the others.

When in the solution if the color shows too bright red, take out the specimen, wipe it, let dry and apply one coat of maroon color made by True Tag Paint Co. of Memphis, Tenn. Add two-thirds turpentine. Repeat the procedure if the color still shows too bright red when replaced in the solution.

THE WATER PAINT METHOD

In using water paints the muscles must also be previously painted with two coats of gelatine.

When the gelatine is dry then the muscles are painted with a first thin coat of water French Carmine as explained for the oil paint. Water Carmine does not come in a tube but in a little cake. This cake should be sawed into little cubes of about one-quarter of an inch, which is the quantity for a teaspoonful of water. The water paint must not be thick. Two thin coats or more are better than a single thick coat.

When the first coat is dry about 8 hours, the second coat of water paint is applied, if necessary. This must also be a very thin coat.

After this second coat the specimen is left exposed to the air until thoroughly dry. If placed in the final permanent aqueous solution, before the paint is dry, the water paint will dissolve. The long time required to dry thoroughly is to the disadvantage of the water paint.

During this drying of the paint, the tendons and bones may become more or less brown from drying, but when placed in the permanent solution they resume their white appearance.

When the paint is dry the preparation is placed permanently in a solution composed of 10 per cent of alcohol, five per cent of formol and eighty-five per cent of filtered water (A 10, F 5).

The solution must be changed or clarified as soon as it becomes slightly cloudy or discolored.

CONCLUSIONS

Sufficient time has not elapsed to state the final result, but my impression is that oil paint preparations kept in 'A 10, will ultimately yield better preparations than when other methods are used.

The appearance of painted preparations in the permanent solutions is satisfactory for educational purposes and for esthetic effect. The artistic skill, judgment and patience of the painter will tell on the final result.

When in course of time the paint shows the least sign of fading or of deterioration, the preparation must be removed from the solution, the old paint wiped off with cheese cloth and the muscles painted again, two thin coats or more. No gelatine is to be applied.

BOOKS RECEIVED

The receipt of publications that may be sent to any of the five biological journals published by The Wistar Institute will be acknowledged under this heading. Short reviews of books that are of special interest to a large number of biologists will be published in this journal from time to time.

AN INTRODUCTION TO NEUROLOGY. C. Judson Herrick, Ph.D., Professor of Neurology in the University of Chicago, 335 pages, 137 illustrations. \$1.75. 1915. Philadelphia and London: W. B. Saunders Company.

Extracts from Preface. There are two groups of functions performed by the nervous system which are of general interest; these are, first, the physiological adjustment of the body as a whole to its environment and the correlation of the activities of its organs among themselves, and, in the second place, the so-called higher functions of the cerebral cortex related to the conscious life. The second of these groups of functions cannot be studied apart from the first, for the entire conscious experience depends for its materials upon the content of sense, that is, upon the sensory data received by the lower brain centers and transmitted through them to the cerebral cortex. Since the organization of these lower centers is extremely complex, and since even the simplest nervous processes involve the interaction and coöperation of several of these mechanisms, it follows that an understanding of the workings of any part of the nervous system requires the mastery of a large amount of rather intricate anatomical detail. * * *

The problems which at present chiefly occupy the attention of neurologists are of two sorts—first, to discover the regional localization within the nervous system of the nerve-cells and fibers which serve particular types of function or, briefly architecture, and second, to discover the chemical or other changes which take place during the process of nervous function, that is, the metabolism of the nervous tissues. The first of these problems is at present further advanced than the second; the larger part of this work is, therefore, devoted to a description of architectural relations. Without a knowledge of these relations, moreover, the problems of metabolism are, in large measure, meaningless. * * *

This little book has been prepared in the hope that it will help the student to learn to organize his knowledge in definite functional patterns earlier in his work than is often the case, and to appreciate the significance of the nervous system as a working mechanism from the beginning of his study.

The structure and functions of the nervous system are of interest to students in several different fields—medicine, psychology, sociology, education, general zoölogy, comparative anatomy, and physiology, among others. The viewpoints and special requirements of these various groups are, of course, different; nevertheless the fundamental principles of nervous structure and function are the same, no matter in what field the principles are applied, and the aim here has been to present these principles rather than any detailed application of them. * * *